TOBACCO INDUSTRY RESEARCH COMMITTEE #181
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: January 6, 1958

1. Name of Investigator: Dr. Benjamin A. Rubin

2. Title: Assistant Professor - Department of Public Health and Preventive Medicine

3. Institution & Address: Baylor University College of Medicine, 1200 M. D. Anderson Blvd., Houston 25, Texas

4. Project or Subject:

An evaluation of the phenomenon of tumor growth enhancement as an assay for carcinogens among the polycylic hydrocarbons and related compounds.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

We have noted (1,2,3) that when carcinogenic polyaromatic hydrocarbons are applied to the skin of certain strains of mice, they are thereafter able to support the progressive growth of a tumor to which they are normally resistant. The non-carcinogenic analogs tested did not have this effect and weakly carcinogenic agents of related structures showed intermediate effects.

Enhancement could be measured in three ways, 1) The fraction of animals showing progressive tumor growth, 2) The extent of growth of the tumors that eventually regress, and 3) The time of persistence of the tumor after controls regress. It was thus possible to devise quantitative, as well as a qualitative estimation of tumor growth enhancement. The plan of procedure for evaluating the usefulness of this effect as an assay method would include the following work.

A group of polycylic hydrocarbons of analogous series would be synthesized, such that there is a known progression of carcinogenicity, both in terms of total effectiveness and in variations of latency in the classical tests. Such series exist in the methylated benzanthracenes, and in the substituted benzacridines (4,5). We have available the services of a synthetic organic chemist who is able to prepare these compounds.

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These series would first be compared in susceptible mice, in a standard fashion in which 0.5 micromols in benzene are dropped on the shaved skin of two groups of six mice each 1) six times in two weeks 2) 18 times in six weeks (every Monday, Wednesday and Friday). Compounds that produce more than 33% of progressive growth in six paintings would be suitably diluted and reapplied for six times at various diminishing concentrations.

Although we have been able in this manner (18 times) to detect a significant effect in the very weak carcinogen 1:2 benzanthracene--if any compound fails to show perceptible effect it will be reapplied for 18 times at increasing levels up to 5.0 micromols per application (solubility permitting).

As far as possible these compounds will then be graded according to the total amount required to produce 33% (estimate 37%) progressive growth. This will be compared with the published (carcinogenicity) values given in the literature for the same substances from classical test methods (5).

In view of the demonstrations that weak carcinogens may inhibit the effect of strong ones, among the polycyclic hydrocarbons, similar quantitive experiments will be carried out, using combinations of agents which have been shown to be "competitive." And we would also test mixtures which have been reported to be additive (6).

We would also determine the quantitive effect in this system of strong carcinogens, such as 3,4 benzpyrene (which is strongly tumor enhancing) alone and in the presence of hydrocarbon mixtures such as composite cigarette smoke condensate (Ecusta) and the benzene-soluble fraction of industrial air pollutants. Effects of each of these mixtures alone would also be found. It might thus be possible to determine whether naturally occurring complex hydrocarbon mixtures can interfere with the detectability of known carcinogens.

This approach gives promise of providing a rapid, economical, and quantitative method of detecting the important carcinogens of the polycyclic hydrocarbon group, even when they exist as minor impurities in complex mixtures. In view of the time requirements and theoretical complexities of present methods for carcinogen testing, it seems advisable to explore the utility and limitations of this enhancement phenomenon. Should it prove to correlate well with classical carcinogenesis studies, it would greatly facilitate the isolation of small active constituents in the complex mixtures obtained from tobacco or air pollution materials.

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References

1. Rubin, B.A., Ida N., and Kirschbaum, A. Growth Enhancement of a Transplanted Mouse Lymphosarcoma. Proc. Cancer Research, 2:143 (1956).
2. Rubin, B.A., Ida N., Studies on the mechanism of growth enhancement of transplanted mouse tumors by carcinogens. Proc. Cancer Res. 2:244 (1957).
3. Rubin, B.A. and Ward, E.B. A new genetic factor affecting tumor transplantation in mice. Genetics 42:392 (1957).
4. Badger, G.M., Chemical Constitution and Carcinogenic Activity in Advances in Cancer Research. Vol 2, Academic Press N.Y. 1954.
5. Greenstein, J.B. Biochemistry of Cancer Academic Press NY 1954.
6. Steiner, P.E. and Falk, H.L. Summation and inhibition effect of weak and strong carcinogenic hydrocarbons. Cancer Res. 11:56 (1951)

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6. Budget Plan:

Approximately $2000 is for chemical synthetic work; $700 for chemicals and supplies and $1800 for animals.

Salaries	0
Expendable Supplies	$4,500
Permanent Equipment	0
Overhead 15%	675
Other	0
Total	$5,175

7. Anticipated Duration of Work:

January 1, 1958 to December 31, 1958

8. Facilities and Staff Available:

We have the use of equipped biological and chemical laboratories and adequate animal quarters. The staff available for this work includes the senior investigator (B.A. Rubin) and Miss Margaret Stitt, research assistant.

9. Additional Requirements:

We will utilize as a consultant, Dr. Thomas Oedene, a synthetic organic chemist, of considerable experience in this field. He has agreed to prepare the needed compounds (not commercially available) for an appropriate fee.

10. Additional Information (Including relation of work to other projects and other sources of supply):

We have another grant in the field of Cancer Immunology from the National Cancer Institute for about $17,500 a year. This is used in part to elucidate the relation of the enhancement phenomenon to the mechanisms of host response to foreign tissue and tumors.

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Signature s/./ B. A. Rubin
Director of Project, B. A. Rubin

Dean, Stanley W. Olson, M.D.

/s./ Alfus O. Johnson
Business Officer of the Institution, Alfus O. Hohnson

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